

ether. Add 20 milliliters of 0.1 *M* potassium phosphate buffer (pH 8.0) and shake. Remove the buffer layer and repeat the extraction with three additional 20-milliliter portions of the buffer. Place the buffer solution in a second separatory funnel and wash with three 30-milliliter portions of ether. Discard the ether washes. Remove an aliquot of the buffer solution and proceed as directed in § 440.80a(b) (1) of this chapter, except § 440.80a(b)(1)(iv) and (ix) of this chapter. If the iodometric chemical assay is used, proceed as directed in § 440.80a(b)(5)(iv)(a) of this chapter, except prepare the sample as directed in § 536.501(a)(1) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Using an aliquot of the buffer solution prepared as directed in paragraph (a)(1) of this section, proceed as directed in § 444.70a(b)(1) through (9) of this chapter, except add sufficient penicillinase to completely inactivate the penicillin present. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as the standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Erythromycin content.* Proceed as directed in § 444.570b(b)(1)(i)(b) of this chapter, except prepare the sample as follows: Place a representative sample (usually approximately 1.0 gram, accurately weighed), in a glass blending jar containing 99 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and 1 milliliter of polysorbate 80. Using a high-speed blender, blend for 2 to 3 minutes. Add 100 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and blend for an additional 2 to 3 minutes. Prepare an intermediate dilution by diluting an aliquot of the filtrate with 0.1 *M* potassium phosphate buffer (pH 8.0), and add sufficient penicillinase to inactivate the penicillin. Then further di-

lute with buffer to give an erythromycin content of 1.0 microgram per milliliter (estimated). Its content of erythromycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.511 Penicillin-streptomycin-bacitracin methylene disalicylate-neomycin ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate-neomycin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter. Its penicillin content is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Bacitracin methylene disalicylate content.* Proceed as directed in § 436.505(a)(3). Its potency is satisfactory if it contains not less than 85 percent of the equivalent number of units of bacitracin that it is represented to contain.

(5) *Neomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.512 Procaine penicillin G-novobiocin-neomycin-dihydrostreptomycin in oil.

(a) *Potency*—(1) *Penicillin G content.* Proceed as directed in § 440.180d(b)(1)(i)(a) of this chapter, using the

novobiocin-resistant strain of *Staphylococcus aureus* (ATCC 12692),¹ except prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 1 percent potassium phosphate buffer, pH 6.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender and make appropriate dilutions, using 1 percent potassium phosphate buffer, pH 6.0. Its content of penicillin G is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Novobiocin content.* Proceed as directed in § 440.180d(b)(3)(i), with the following exceptions:

(i) Prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1M potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6) to give a final concentration of 0.5 microgram novobiocin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 50 WX-4, Na⁺ type 200-400 mesh, sufficient to make a total concentration of 2 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of novobiocin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Neomycin content.* Proceed as directed in § 436.517(b)(1) of this chapter, using the *Staphylococcus epidermidis* (ATCC 12228)¹ procedure, except:

(i) Prepare the sample as follows: Place the equivalent of one dose of

sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1M potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1M potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram neomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 1-X8, Cl type 200-400 mesh, to make a total concentration of 1 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Dihydrostreptomycin content.* Proceed as directed in § 436.105 except prepare the sample by placing the equivalent of one dose in a blender, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1M potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1M potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram dihydrostreptomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

[39 FR 18944, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976]

§ 436.513 Chlortetracycline troches; tetracycline hydrochloride troches.

(a) *Potency.* If it is tetracycline hydrochloride proceed as directed in § 446.81a(b)(1) of this chapter and if it is

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.